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Thyroid Economy—Regulation, Cell Biology, Thyroid Hormone Metabolism and Action: The Bianco Special Edition: Metabolic Effects of Thyroid Hormones

Thyroid Hormone and Adipocyte Differentiation

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Thyroid hormones act as pleiotropic factors in many tissues during development, by regulating genes involved in differentiation. The adipose tissue, a target of thyroid hormones, is the main place for energy storage and acts as a regulator of energy balance, sending signals to keep metabolic control. Adipogenesis is a complex process that involves proliferation of preadipocytes and its differentiation into mature adipocytes. This process is regulated by several transcription factors (CCAAT/enhancer-binding proteins [C/EBPs], peroxisome proliferator-activated receptors [PPARs]) that act coordinately, activating adipocyte-specific genes that will provide the adipocytic phenotype. Thyroid hormones regulate many of those genes, markers of differentiation of adipocytes, those involved in lipogenesis, lipolysis, and thermogenesis in the brown adipose tissue (BAT). Triiodothyronine (T3) actions are achieved either directly through specific thyroid response elements (TREs), by regulating other key genes as PPARs, or through specific isoforms of the nuclear T3 receptors. The availability of T3 is regulated through the deiodinases D3, D2, and D1. D3 is activated by serum and mitogens during proliferation of preadipocytes, while D2 is linked to the differentiation program of adipocytes, through the C/EBPs that govern its functionality, providing the T3 required for thermogenesis and lipogenesis. The relationship between white adipose tissue (WAT) and BAT and the possible reactivation of WAT by activation of uncoupling protein-1 (UCP1) is discussed.

Introduction

Thyroid hormone actions are pleiotropic, involving the regulation of many physiological systems. Their actions are especially important during development. One of the best-studied actions of thyroid hormones is the regulation of the metamorphosis of amphibians (1–4). Thyroid hormones regulate the growth and maturation of many organs and tissues during the fetal and neonatal life (5,6) as well as other specific organs like the cochlea or specific regions of the retina (7,8). Many tissues are regulated by thyroid hormones up to their complete development, including actions on groups of genes involved in the differentiation program. The supply of thyroid hormones is also regulated in a time-specific way and in precise areas of the brain, through the deiodinases D2 and D3, as demonstrated in the human fetal brain (9) or in the cochlea (10). During the adult life, thyroid hormones regulate the metabolism and function of many tissues, such as liver, heart, skin, muscle, or adipose tissue.

The adipose tissue is one of the targets of thyroid hormones. The adipose tissue is specialized in the transport, synthesis, storage, and mobilization of lipids. Its main function is the storage of energy in the form of triglycerides, and it constitutes a reservoir of energy to be used in times of caloric deprivation.

There are two types of adipose tissue: white adipose tissue (WAT) and brown adipose tissue (BAT), which have distinct features. The WAT is considered as a reservoir of lipids and energy and morphologically is characterized by a large lipid droplet that fills the cellular space. WAT is distributed in many different anatomical locations: subcutaneous and visceral (omental) fat, which have different lipolytic sensitivity and response to drugs and hormones and represent a different health risk (insulin resistance and metabolic syndrome) (11). But we can find also fat deposits covering other organs such as the heart, kidney, or the sexual organs (perirenal and perigonadal depots). To which extent this adipose tissue is pure WAT is under discussion because some of these locations are reminiscent of the primitive BAT locations. WAT in

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humans is a large and disperse tissue along the body accounting for about 15% of total body weight in control subjects, a percentage that can increase up to 40% in obese patients.

The BAT is a tissue specialized in the adaptation to cold by adaptive or facultative thermogenesis or to rich diets (protection against obesity). BAT is found predominantly in hibernating animals, small rodents, and newborns (12). The main function of BAT is to dissipate energy instead of storing it. The dissipation of energy is accomplished by the BAT-specific protein, uncoupling protein-1 (UCP1), which generates heat by uncoupling the respiratory chain. BAT is found in small, disperse locations in the body, protecting heart, kidneys, aorta, and other organs. It is highly innervated and irrigated. Morphologically BAT is characterized by multilocular lipid droplets and abundant mitochondria, which increase under cold exposure. The capacity of BAT to dissipate energy is considered as a possible tool against obesity in case it could be reactivated.

Proliferation and Differentiation of Adipocytes

The adipose tissue was considered for many years only as a reservoir of lipids, a place for lipid storage and mobilization, but during the last 15 years there has been a burst of information related to adipose tissue, its regulation, its secretory function, the adipocyte-specific genes, and the signaling pathways altered in pathological situations, leading to a better knowledge of this tissue and the main features of its functional unit, the adipocyte.

The research on adipose tissue was hampered for years due to the difficulties inherent to its high lipid content and the lack of suitable cell culture models for its molecular study. The establishment of preadipose cell lines, derived from NIH 3T3 fibroblasts (3T3-L1 and 3T3-F442 cell lines), allowed the study of the differentiation of the adipocyte. The pioneering studies from the groups of Spiegelman and Lane have thrown light on the molecular biology of the adipocyte (13,14). They initially identified a series of lipogenic and glycolytic enzymes that increased several fold during the process of the adipocyte

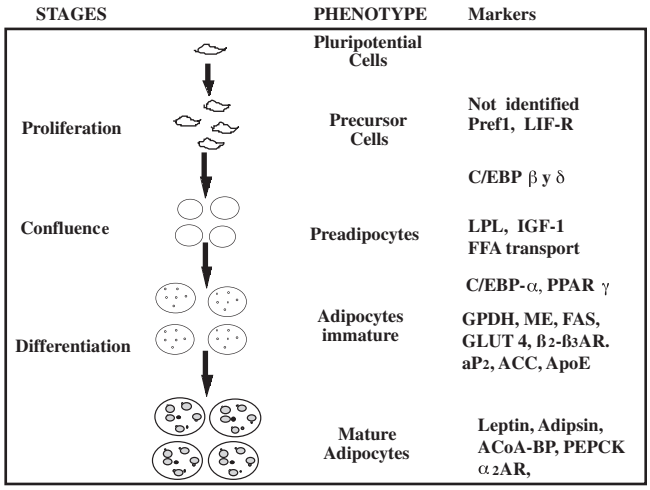

FIG. 1. Different stages of white adipocyte differentiation are shown, from precursor cells to mature adipocytes. The enzymes and proteins expressed at the different stages of differentiation are shown in the right column. Modified from Ailhaud *et al*. (21).

differentiation, by measuring its activity, protein levels, and mRNAs. Among these markers were the fatty-acid-binding protein aP2 (15), glycerophosphate dehydrogenase (GPD) (13), acetyl-CoA carboxylase (ACC) (16), the stearoyl-CoA desaturase (SCD) (17), the fatty acid synthetase (FAS) (16), the lactic dehydrogenase (LDH), lipoprotein lipase (LPL), malic enzyme (ME), phosphoenolpyruvate carboxykinase (PEPCK), and some new genes as adipsin (18,19) and adipoQ (20), now called adiponectin. Among those proteins some are early markers of adipocyte differentiation and others appear later on, as seen in Figure 1, and as reviewed by Ailhaud *et al.* (21). The C/EBPs transcription factors were early identified by Lane and coworkers as the ones governing the process of adipocyte differentiation (22,23).

The adipocyte is the functional unit of the adipose tissue. This cell, specialized in the storage of lipids, acquires its full capacity after a complex process called adipogenesis, which involves proliferation from preadipocytes or mesenchymal-type cells and a coordinated process of differentiation that confers the adipocyte the full capacity to accomplish its specialized functions.

Proliferation of preadipocytes

The preadipocyte is a mesenchymal-type cell that derives from pluripotential stem cells, which become predetermined to be preadipocyte. The factors that trigger these first steps from pluripotential stem cells into preadipocytes have not been elucidated. Osteoblasts, myoblasts, and adipocytes derive from a common mesenchymal precursor cell (24,25). Mesenchymal stem cells seem to differentiate into adipocytes under PPARγ2 activation (26), but the intermediate steps that trigger the activation are far from being identified. Leukemia inhibitory factor (LIF) has been proposed as one of the markers of these initial steps, inducing the adipocytic phenotype together with PPARγ2. The preadipocyte factor 1 (Pref-1, also called *Dlk1*) is an imprinted gene found only in preadipocytes and is a potent inhibitor of adipogenesis (27). Pref-1 activates MEK-extracellular signal-regulated kinase (ERK) phosphorylation (28–30) and is a marker of preadipocyte stage (Fig. 1). Certain *HOX* genes display a specific expression in WAT, and the expression of four *HOX* genes appears to discriminate between WAT and BAT (31). A recent study using microarrays has identified Pref-1 as one of the markers of the proliferative stage in brown preadipocytes, while C/EBPδ and Necdin are markers of proliferating brown and white preadipocytes (32). Necdin is a transcriptional regulator that would inhibit the activation of the PPARγ1 promoter (33).

The preadipocytes, also called "precursor" cells or mesenchymal-type cells, present in the stroma-vascular fraction of the adipose tissue, even in adult animals, though they are present in smaller number. The identification of these precursor cells has been very important for the research in adipose tissue because it has allowed to establish primary cultures of preadipocytes, which proliferate and differentiate in culture (34). The proliferation of preadipocytes occurs under the stimulation of several growth factors present in serum, mainly those from the family of the fibroblast growth factors (FGFs). The proliferation of brown preadipocytes is highly stimulated under cold exposure and was first studied *in vivo* using tritiated thymidine, establishing the β-adrenergic nature of the process of proliferation (35–37), while insulin

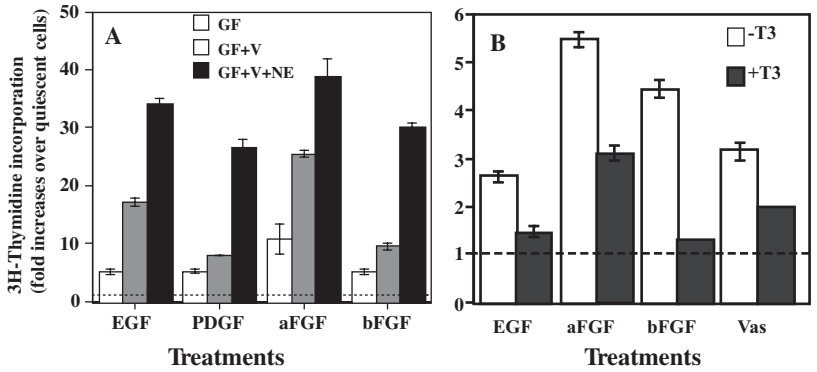

FIG. 2. Proliferation of brown preadipocytes. (**A**) We show the increases in DNA synthesis in brown preadipocytes using different growth factors (EGF, PDGF, aFGF, and bFGF), alone or combined with Vasopresin (V) and/or norepinephrine (NE). (**B**) Antimitotic effect of triiodothyronine (T3) on DNA synthesis induced by growth factors, measured as thymidine incorporation (40).

was proposed as a mitotic factor for white adipocytes (38). Studies in primary cultures of brown preadipocytes demonstrated that the β1 adrenergic stimulation increases DNA synthesis (39). Our studies showed that norepinephrine (NE), a poor mitogen itself, increases the mitogenic action of serum, growth factors, and the neuropeptide vasopressin (40) (Fig. 2A). Therefore, NE has an important role in brown adipocyte proliferation, besides its role in increasing thermogenesis, specifically UCP1 mRNA expression.

The role of thyroid hormones during the proliferative stage of adipocytes seems to be antimitogenic, as the addition of triiodothyronine (T3) inhibits the mitogenic activity of bFGF and aFGF in brown preadipocytes (41) (Fig. 2B). In fact, D3 activity and mRNA are highly induced by growth factors in primary cultures of brown adipocytes (42,43), suggesting that a reduction of T3 levels is physiologically important under mitogenic stimulation. Large D3 increases (activity and mRNA) are also observed when serum is added to primary cultures of brown adipocytes (44) (Fig. 3). This led us to propose that D3 acts as a marker of proliferation in brown preadipocytes. As opposite, little D2 activity is observed during the proliferative stages, pointing to a differential role of both deiodinases, D3 being present during proliferation and D2 having a role during the differentiation stages.

Proliferation studies using white preadipocytes are scarce, although serum clearly stimulates DNA synthesis and proliferation in white preadipocytes in primary cultures (unpublished results). The identification of the specific growth factors responsible for the proliferation of white preadipocytes requires further investigation. It has been postulated that the proliferation of white preadipocytes requires only T3, insulin, and transferrin in serum-free medium (45,46). However, evidence indicates that the preadipocytes of obese people secrete mitogenic factors that induce a higher proliferation rate than the conditioned media derived from preadipocytes from control subjects (47). Macrophage-secreted factors have also been proposed as mitogens in human preadipocytes (48), but the specific growth factors or adipokines involved have not been identified. FGF10 has been proposed as one of the mitogens for WAT, because the development of WAT in FGF10$^{-/-}$ mouse embryos is greatly impaired due to a decreased proliferative activity of WAT, indicating that FGF10, not C/EBPα, is required for the proliferation of white preadipocytes (49). Adipose tissue is a source of growth factors that could stimulate proliferation, such as insulin growth factor I (IGF-I), IGF-binding proteins, tumor necrosis factor α (TNF-α), angiotensin II, and macrophage colony-stimulating factor (MCSF) (50).

Differentiation of the adipocytes: the role of transcription factors

The process of adipocyte differentiation was first studied as a unique process in preadipose cells lines (3T3-L1 and

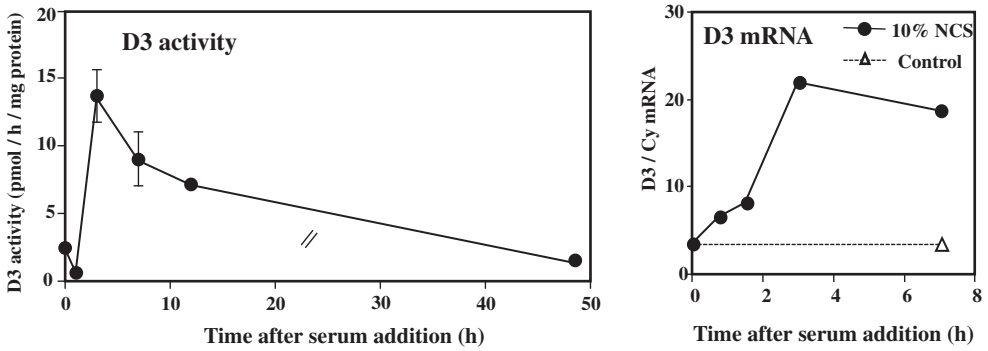

FIG. 3. Time-course induction of D3 activity (left) and D3 mRNA (right) using 10% newborn calf serum (NCS) in brown adipocytes (44).

3T3-F442). Differentiation was usually induced by adding dexamethasone and agents that increase cAMP (IBMX). T3 was often included in the "differentiation cocktail." In this way it is difficult to predict if the effects observed are due to the process of differentiation itself or to the action of the hormones added. During the adipose differentiation of 3T3-F442A or 3T3-L1 cells, there is a large increase in the transcription of specific genes and increases in the synthesis of numerous proteins, including the lipogenic enzymes GPD, FAS and ME, and many others as described above (13,16). There is a sequential activation of a whole set of proteins and enzymes that act as differentiation markers, with different timings for their transcriptional increases (Fig. 1). Early markers are LPL or IGF-1, preceded by the families of transcription factors C/EBPs and PPARγ. They are followed by many lipogenic enzymes and other proteins: GPD, ME, FAS, aP2, Glut4, ACC, and the β- adrenergic receptors, among many others. Later markers of differentiation are leptin, adipsin, PEPCK, the α-2 adrenergic receptors, and others (21). Recently, studies using genomic techniques (microarrays) (51) have shown that the cellular programs associated with adipocyte differentiation are more complex than previously thought as derived from the changes observed *in vitro* in the mentioned cell lines and the changes in gene expression associated with adipocyte development, revealing that adipocyte differentiation *in vivo* and *in vitro* are quite different. Those studies confirmed many genes that had been previously reported to increase during differentiation of cell lines, for example, C/EBPδ, C/EBPβ, C/EBPα, aP2, adipsin, adiponectin, LPL, HSL, SCD1, GPD, and so on. Other genes were induced later on, as PEPCK, β3-AR, PFKI, IGF-II, and so on. But several genes were expressed only in cell lines *in vitro* or only cells derived from tissues *in vivo*. These data suggest that one or more transcriptional programs are activated exclusively *in vivo* to generate the adipocyte phenotype.

Therefore, the differentiation of preadipocytes into adipocytes is achieved by the coordinate activation of transcription of several adipose-specific genes (52). The adipocyte differentiation follows a common adipogenic transcriptional pathway, regulated by the transcription factor families of C/EBPs and PPARs. All the adipogenesis is regulated by the sequential activation of these transcription factors and the nuclear proteins (coactivators or inhibitors) that regulate them.

The C/EBPs are transcription factors of the basic leucine zipper family. Several members of the C/EBP family (C/EBPα, C/EBPβ, and C/EBPδ) have tissue-specific expression patterns and recognize a common DNA-binding element. C/EBPα is expressed in brown and white adipose tissues, placenta, and liver. C/EBPα is a master regulator of adipose tissue development. C/EBPα is required for the differentiation of 3T3-L1 preadipocytes, and when overexpressed, is able to trigger the differentiation of 3T3-L1 preadipocytes. It also works as an antimitotic signal inducing proteins associated with growth arrest (GADD45 and p21) (53). The induction of C/EBPα in preadipocytes increases the expression of several adipocyte-specific genes (aP2, Glut4) and the accumulation of triglycerides (53,54).

The C/EBPβ and possibly C/EBPδ transcription factors are expressed earlier than C/EBPα in the differentiation program and activate transcriptionally C/EBPα, triggering the process of differentiation, while PPARγ and C/EBPα induce the differentiation from preadipocytes into adipocytes, followed by the adipo-specific gene expression. Most of those genes (SCD1, aP2, S14, PEPCK, and Glut4) have C/EBP-binding domains in their promoters and are activated in a coordinated program during adipogenesis (22), and we cannot forget to include two important ones for the differentiation of brown adipocytes, UCP1 and D2, which we will comment later on. C/EBPβ and C/EBPδ increases precede those of C/EBPα. During the development of BAT during fetal life, C/EBPβ and C/EBPδ increases also precede C/EBPα expression (55).

The study of the mice with a deletion in the C/EBPα gene (C/EBPα knockout mice) throws some light on the function of this transcription factor. The knockout mice die shortly after birth due to severe hypoglycemia and defective hepatic glycogen storage and gluconeogenesis (56). C/EBPα knockout mice do not have WAT and showed a great reduction in BAT depots and a very low UCP1 mRNA expression. This shows that C/EBPα is essential for the survival of the mice and for the developmental program of liver and adipose tissue. The status of BAT was examined in the C/EBPα knockout mice (57). UCP1 expression was very low; adipogenesis was impaired; the size, number, and function of the mitochondria were reduced. The expression of thyroid hormone receptors (THRs) and PGC1 (the coactivator of PPARγ) was delayed (57). We found that BAT D2 activity was very low (Fig. 4), together with low T3 levels in BAT; this indicates that C/EBPα is critical to maintain thyroidal status in BAT. Indeed, D2 has C/EBP elements in its proximal promoter. This suggests that D2 plays a crucial role in the differentiation program of fetal BAT and in some way is linked to it for its full functionality, possibly because T3 is absolutely necessary for its function.

Neonatal hypothyroidism decreases C/EBPα and C/EBPβ expression in liver, but not in BAT (58), and several TREs have been identified in the C/EBPα promoter (59). In the PEPCK gene, a relationship between C/EBPs and TREs has been described, as in this gene the activation of C/EBPs is required for a functional TRE (60).

Besides the participation of the C/EBPs, adipocyte differentiation is a process regulated also by the PPAR family, specifically by PPARγ. PPARs belong to the family of nuclear

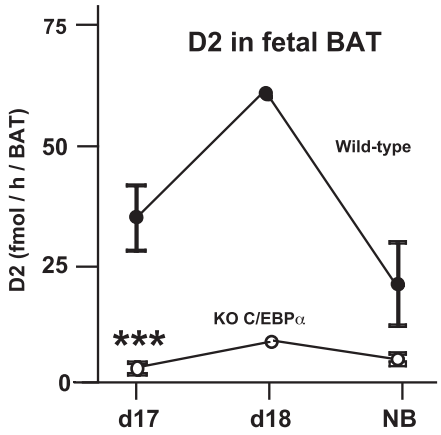

FIG. 4. D2 activity in fetal (d17 and d18) and neonatal (NB) BAT of CCAAT/enhancer-binding protein (C/EBP) knockout mice and wild-type mice (57). *** refers to $p<0.05$ vs wild type.

receptors that act as transcription factors regulating changes in gene expression in response to nutritional stimuli and controlling lipid metabolism. PPARs family controls the metabolism of fatty acids, which are natural ligands that activate PPARs, especially arachidonic acid and its metabolites. PPAR family has several members: PPARα (activated by fibrate and regulates β-oxidation, lipid catabolism, and inflammation), PPARδ, and PPARγ, which is quite specific of adipose tissue. PPARs form heterodimers with the X receptor of retinoic acid (RXR), activating the PPAR response elements (PPREs) (DR-1, 6-base pair direct repeats of the sequence RGGTCA spaced by one base) present in the promoter of specific target genes. Most of the genes mentioned above have PPREs in their promoters: aP2, FAS, PEPCK, LPL, SCD, and so on. Those PPRE-binding sites can bind different isoforms in different tissues, for example, for the LPL gene, the PPARα isoform in the liver and the PPARγ isoform in the adipose tissue. There is abundant information that indicates that PPARs play important roles not only in adipogenesis but also in inflammation, atherogenesis, glucose homeostasis, and cancer.

All the adipogenesis is regulated by the activation of the PPARγ (61), which in turn is regulated by C/EBPα and possibly by C/EBPβ (Fig. 5). The ectopical expression of PPARγ is able to induce fibroblastic cell lines to differentiate into adipocytes under the appropriate stimuli of agonists of PPARγ (thiazolidinediones) (61).

As the mice with targeted deletion of PPARγ were lethal, several strategies have been used; the main one was to use the adipose-specific PPARγ knockout mice. PPARγ knockout mice presented several alterations with contrasting results. Some studies have showed a reduced fat formation, and protection against obesity and insulin resistance with lipodystrophy (62). The mice with targeted deletion of PPARγ2 have insulin resistance, indicating that PPARγ2 is necessary for the maintenance of insulin sensitivity (63).

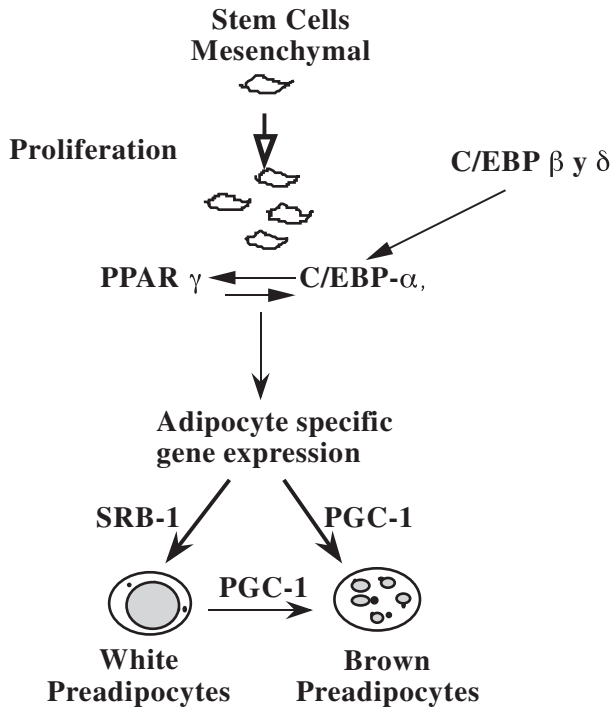

FIG. 5. Transcription factors that regulate the differentiation of adipocytes. The role of the coactivator of PPARγ, PGC1, is also depicted. Modified from Puigserver and Spiegelman (65).

The specific coactivator of PPARγ, PGC1, was identified in 1998 (64). PGC1 greatly increased under cold exposure in BAT. PGC1 increased the transcriptional activity of PPARγ and THR on the UCP1 promoter. As the ectopic expression of PGC1 in white adipocytes activated the expression of UCP1 and mitochondrial enzymes of the respiratory chain, PGC1 was considered as the true transcriptional activator of BAT and adaptative thermogenesis, as well as a marker of brown adipocytes (64). Later on, it has been identified as fundamental for several processes like hepatic gluconeogenesis, heart function in which mitochondriogenesis is very important, and inflammation (65–67).

The mice with targeted deletion of PGC1α present several abnormalities in muscle, hepatic steatosis, increase in body fat, diminished mitochondrial number and respiratory capacity, and abnormal cardiac function (68). The importance of the coactivator of PPARγ, PGC1, is explained in one of the previous chapters (Chapter 8).

Regulation of gene expression in adipocytes by T3 and its nuclear receptors

T3 regulates adipogenesis and processes related to them as lipogenesis and lipolysis both *in vivo* and in cultured adipocytes (21,69). Indeed, all the isoforms of THRs, TRα1, TRα2, and TRβ1, are expressed in white and brown adipocytes and in WAT and BAT, TRα1 being the predominant TR isoform (70–73). T3 and other hormones regulate the different TR isoforms. Two main approaches have been used to discriminate between the effects of both TR isoforms: a pharmacological approach, using ligands specific of the TRβ isoform, and a genetic approach, using knockout and knockin mice.

Selective agonists of the TRβ isoform have been used to increase metabolic rate and lower cholesterol, triglycerides, lipoproteinA, and body weight without affecting heart rate (74). The TRβ1 agonist GC-1 was used to stimulate UCP1, with no effect in regulating body temperature, therefore discriminating two isoform-specific actions of T3 in BAT (75).

To identify the specific actions of the α- and β-TR isoforms, several TR knockout and knockin mice were generated (76). Although the first knockout mice studied did not show phenotypic abnormalities concerning adipose tissue metabolism or alterations in energy balance, recent work has identified several phenotypes related to adipose tissue and energy balance, due to point mutations or negative dominant mutations in the TRs. TRα seems to be required for proper thermogenesis (77), while TRβ regulates cholesterol metabolism (78). Mice devoid of all TRs have decreased body temperature and basal metabolic rate and are cold intolerant due to insufficient heat production (79). These null TR mice show growth retardation and delayed skeletal maturation together with an increased amount of fat and increases in several adipocyte-specific genes (80). Moreover, certain mutations in the THRs, specifically the P398H mutation in the TRα gene, induce visceral adiposity, hyperleptinemia, a fourfold increase in body fat, increased basal glucose and insulin, and impaired lipolysis in male mice (81). Many genes of the lipogenic and lipolytic pathways were decreased. Adaptative thermogenesis was also reduced. This mutation reduces the

PPARα binding to PPREs, interfering with PPARα signaling (82). Recently, the role of unliganded TRs, acting as aporeceptors and exerting opposite transcriptional effects, has been investigated using a dominant negative mutation (R384C) in the TRα1 gene that causes a 10-fold reduction in the affinity to T3 (83). These mice were hypermetabolic and had reduced fat depots, hyperphagia, and resistance to diet-induced obesity, together with an induction of the genes involved in glucose handling and lipid mobilization and β-oxidation. The alterations are reversed by increases in T3 levels. Thus, TRα1 aporeceptor is involved in metabolic homeostasis. Similar results are found in other heterozygous mice with a dominant negative mutation of the TRα1 (PV/+), in which WAT is reduced, as well as the expression of PPARγ signaling, affecting adipogenesis (84).

Many of the genes involved in the differentiation program of adipocytes are regulated by T3. The list of genes includes GPD, ME, PEPCK, S14 (85), FAS (86), GLUT4, and LPL, among many others (87–89). Many of those genes are directly regulated by T3 through the TREs present in their promoters (90–92). Some enzymes such as α-glycerophosphate dehydrogenase (α-GPDH) and ME have been used extensively to check the thyroidal status in experimental animals (usually in liver). Many groups accomplished the study of the T3 actions at the promoter level, identifying the functional TREs and its interactions with other members of the family of nuclear receptors, such as PPARs, retinoic acid, or with insulin response element (IRE) and cAMP response elements (CREs). Usually there is a strong interaction among all these elements and the coactivators that regulate them, as have been described in several genes as UCP1, ME, ACC, and others. We studied the regulation of ME and S14 by T3 in brown preadipocytes in culture (93–95). ME is a lipogenic enzyme that plays a key role in differentiation, and S14, a T3-responding gene and abundant in lipogenic tissues, is now considered a possible transcription factor in lipogenesis. These two genes increase during differentiation of preadipocytes, and its natural progression increases in the presence of by T3. The action of T3 is both transcriptional and stabilizing the mRNA, and it is synergic with the action of insulin. The effect of NE and retinoic acid was also examined (94). A detail study of the effect of T3 on lipid synthesis is presented in the next chapter.

T3 actions on UCP1 and thermogenesis: role of D2

When working with 3T3-L1 preadipocytes (white), the differentiation of adipocytes is measured by its lipogenic capacity and the capacity for lipid accumulation, measuring the increase in lipid droplets and increased gene expression in lipogenic enzymes. But in BAT, the differentiation process involves in addition the acquisition of the full thermogenic capacity, as measured by the full expression of UCP1 and the activation of the process of mitochondriogenesis.

As described above, the function of BAT is to generate heat when the demands increase under cold exposure. The generation of extra heat is accomplished by the mitochondrial UCP1 that uncouples the oxidative phosphorylation. BAT is activated by the sympathetic nervous system (SNS), via the NE released from the nerve endings (96). The binding of NE to the adrenergic receptors and the activation of adenylate cyclase increases cAMP, that activate lipolysis, increasing free fatty acids (FFA) which activates UCP1 (12). The thermogenic capacity of BAT is determined by the amount of UCP1. UCP1 is transcriptionally activated shortly after NE or cold exposure (97,98). It has been demonstrated that T3 amplifies the adrenergic stimulation of UCP1 (98,99). Moreover, in thermoneutral conditions, like during the intrauterine life, T3 is required for the expression of UCP1 mRNA, and euthyroidism is required during the first postnatal days for the increases in UCP1 mRNA (Fig. 6) (100,101). In cultures of rat brown adipocytes, it is clear that T3 is an absolute requirement for UCP1 adrenergic increases, participating in the stabilization of mRNA transcripts (102). T3 itself is also able to induce the transcription of UCP1 in fetal rat brown adipocyte in primary culture (103).

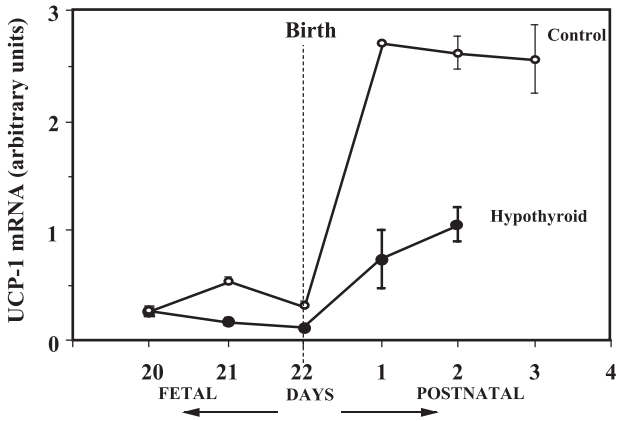

FIG. 6. Expression of uncoupling protein (UCP1) in fetal and neonatal BAT obtained from control and hypothyroid fetus and litters. Hypothyroidism was induced using methylmercapto-imidazol (MMI) in the drinking water (100).

The study of the UCP1 promoter provided evidence for the presence of CREs (104–106) in the proximal promoter, and an enhancer element was identified that contained several TREs (92,107), some retinoic acid response elements (RAREs) (108,109), and a PPRE (110). There is clear cross-talk among these nuclear receptors and its coactivators for its binding to the UCP1 promoter. In addition, there are negative regulators of the expression of UCP1: serum, mitotic signals involving the activation of c-jun (106), and insulin. Other hormonal treatments (glucocorticoids or sexual hormones) can also modulate UCP1 expression.

Recently, we have examined the role of Triac in cultured brown adipocytes (111). Triac, which binds better than T3 to the TRβ isoform, is 10–50-fold more potent than T3 in increasing the adrenergic induction of UCP1 and D2, as well as LPL mRNA. The role of Triac was studied in rats (112). Triac was again more potent than T3 (in terms of doses and concentrations) in the stimulation of UCP1, LPL, and leptin, and at low doses, induced ectopic UCP1 expression in WAT (112).

The adrenergic input also increases D2 deiodinase in BAT (113), causing a marked increase in T3 in BAT, suggesting that T3 plays an important role in this process. It was also demonstrated that the intracellular conversion of thyroxine to T3 was required for the full thermogenic function of BAT (114). This is also true for the adrenergic stimulation of D2 (115,116), which does not take place unless T3 is present. It is evident that D2 participates in the formation of BAT, as evidenced in the experiments described above in C/EBPα knockout mice

(57), where UCP1 expression and D2 activity are blunted, as well as other markers of mitochondriogenesis. D2 has also been implicated in the process of lipogenesis under adrenergic stimuli (89). The analysis of the D2 knockout mice reveals that there is a hyperadrenergic stimulation, which compensates for the lack of T3 production in BAT. Lipogenesis is unable to provide the high FFA levels required during cold exposure, resulting in an impaired adaptative thermogenesis (117).

The importance of deiodinases in the differentiation of white adipocytes is still poorly studied. It is evident that there is a role in lipogenesis and in the expression of genes involved in the differentiation program. In addition D1 is found in WAT (118), but its precise role has not been studied. To what extent the role of D2 and D1 is different from that studied in brown adipocytes remains to be seen.

The distinction between white and brown adipocytes: reinduction of WAT into BAT

There are important questions on the relationships between white and brown adipocytes. In the early 1980s, comparative studies using primary cultures of brown and white adipocytes (34) established that precursor cells from epididymal fat (pure WAT) and from interscapular BAT differentiate into white and brown adipocytes, respectively, with different phenotypes, characteristics, and regulation. The work done for more than 20 years using these primary cultures confirms the hypothesis that precursor cells are already committed to become brown or white adipocytes. The question whether BAT and WAT derive from the same or different preadipocyte precursor cells is still under discussion. It is not known if the undetermined mesenchymal stem cells retain some myogenic or chondrogenic potential, as many reports have proposed. Recently, Timmons *et al*. have analyzed this problem using microarrays to study both preadipocytes in culture (32). They have found a myogenic signature in brown preadipocytes, not found in white adipocytes, in which they have found a transcription factor, Tcf21, which suppresses myogenesis. They have found genes only expressed in brown adipocytes or only in white adipocytes, as well as markers of differentiation and proliferation for white and brown preadipocytes and genes implicated in human obesity (32).

There is a growing interest in this field as brown adipocytes are associated to increased energy expenditure and the conversion of white adipocytes into brown adipocytes is sought as a strategy to fight obesity. Indeed under extreme cold exposure, a reactivation of BAT adipocytes in inguinal WAT is observed, and this type of WAT was called convertible adipose tissue due to its capacity to revert to BAT (119). Many attempts have been made in this sense, and there are an increased number of experimental models in which a reactivation of WAT into BAT is observed. The increase in UCP1 expression is the golden rule to assess such a conversion of WAT into BAT. This fact has been observed using some drugs and also in mice with targeted deletion of a certain genes. Beta 3 adrenergic agonists are able to induce UCP1 in muscle, and ectopic BAT present in muscle provides a mechanism against weight gain (120). Brown adipocytes are also found in WAT (121–123). The same effect is observed using models of hyperleptinemia that depletes fat stores in rats (124,125). Tungstate has a similar effect with reactivation of energy metabolism (126), and we observed that Triac at low doses induce UCP1 expression in inguinal WAT in rats (112). In mice with targeted deletion of the corepressor RIP140 (127), UCP1 expression is increased and the mice are lean and resistant to diet-induced obesity.

Conclusion

In summary, adipogenesis is a complex process that involves activation of transcription of many genes and enzymes, in a cascade of events regulated by transcription factors that rule the process of differentiation (C/EBPs, PPARs, and PGC1). T3 regulates many of the enzymes involved in this process, either directly or through the interaction with other coactivators, such as PPARs. The deiodinases play a role by providing the T3 necessary for this process or limiting its levels. D3 is stimulated by proliferation, while D2 plays a crucial role in the development of the tissue, in thermogenesis and lipogenesis. The reactivation of WAT depots into depots containing BAT adipocytes could be achieved by some drugs and is observed in some models.

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